Turkish Biochemical Society

Guideline for Complete Blood Count in Medical Laboratories: Effects of Preanalytical Parameters

Prepared by the Turkish Biochemical Society Preanalytical Phase Working Group.

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CONTENT

Abbreviations 8

1. INTRODUCTION 9

2. GENERAL INFORMATION 9

2.1. Structure and Cellular Components of Blood 9

2.2. Development of Complete Blood Count Method 14

3. PRINCIPLES OF THE MEASUREMENT IN AUTOMATIC BLOOD COUNT DEVICES 16

3.1. Impedance Method in Blood Count (Coulter Method) 16

3.2. Flow Cytometry Method in Blood Count 18

3.3. Calculation of Complete Blood Cell Count Parameters 20

4. VENOUS BLOOD COLLECTION FOR COMPLETE BLOOD COUNT 25

4.1. Preparing the Patient 25

4.1.1. Posture 25

4.1.2. Exercise 25

4.1.3. Circadian Rhythm 25

4.1.4. Stress 25

4.1.5. Diet 25

4.1.6. Smoking 26

4.2. Blood Sampling Equipment Used in Complete Blood Count 26

4.2.1. Blood Sampling Tubes 26

4.2.2. Tube Additives: Anticoagulants 26

4.2.3. Insufficient Sample Volume 27

4.2.4. Blood Sampling Needle 28

4.3. Venous Sampling for Complete Blood Count 29
4.3.1. Blood Sampling Staff 29
4.3.2. Order of Draw For Edta Tubes 29
4.3.3. Mixing The Edta Tube 30
4.3.4. Edta-Dependent Pseudothrombocytopenia 30
4.3.5. Effect of Blood Sampling Procedures 31
4.3.6. Blood Volume 31

5. SAMPLE TRANSPORTATION 33

6. SAMPLE STORAGE 33

6.1. Time Period of Complete Blood Count and Conditions of Sample Storage .. 33

7. CRITERIA OF SAMPLE REJECTION 33

7.1. Clotted samples 34
7.2. Hemolysis 35
7.3. Icterus (Bilirubinemia) 36
7.4. Lipemia 37
7.5. Cold Agglutinins 39

Referances 41

Abbreviations

ADP: Adenosine diphosphate

EDTA: Ethylenediaminetetraacetic acid ($C_{10}H_{16}N_2O_8$)

ESR: Erythrocyte sedimentation rate

HCT: Hematocrit

HGB: Hemoglobin

K_2EDTA: Dipotassium EDTA

K_3EDTA: Tripotassium EDTA

LD: Lower detection limit

MCH: Mean corpuscular hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

MCV: Mean corpuscular volume

MPV: Mean platelet volume

Na_2EDTA: Disodium EDTA

NADPH: Nicotinamide adenine dinucleotide phosphate

NK: Natural killer

PCT: Plateletcrit

PDW: Platelet distribution width

PET: Polyethylene terephthalate

PLT: Platelet

PMN: Polymorphonuclear neutrophils

PP: Polypropylene

RBC: Red blood cell

RDW: Red cell distribution width

UD: Upper detection limit

VLDL: Very low density lipoprotein

WBC: White blood cell

1. INTRODUCTION

As technology progresses, automatic analyzers substitute manual procedures and reduce analytical errors. However, as the number of test orders in hospitals and correspondingly as the number of sample accessioning in medical biochemistry laboratories increase, the preanalytical phase of the total continuum becomes more inclined to errors. At present, the vast majority of errors affecting test results occur in the preanalytical phase (1). Among the tests studied, one of the most measured tests is the complete blood count (CBC). Therefore, CBC is one of the tests which are numerically affected by the preanalytical errors (2). Management of the preanalytical phase is difficult due to the need for participation of divisions other than the laboratory. Knowledge about variables impacting CBC test has great importance for preventing preanalytical errors. Developing and complying with the instructions related to this issue are included in the responsibilities of the laboratory management. This reference document is prepared with the purpose of performing a useful, practical and applicable guideline for all medical laboratory staff, nurses, phlebotomists, students and researchers.

2. GENERAL INFORMATION

2.1. Structure and Cellular Components of Blood

Blood is a life-sustaining fluid. Blood, also defined as a tissue, is a crucial tool in evaluating the health status of the individual for it circulates almost all over the tissues. Blood has two essential components;

1. Cellular components,
2. Plasma.

The blood count is the most frequently used measurement method for assessing the blood's cellular components.

Red Blood Cells (RBC, erythrocytes): Red blood cells are akaryocytes in the shape of a concave disc. Its diameter is 6.2 - 8.2 µm in average and its volume is 90 fL on average (Figure 1).

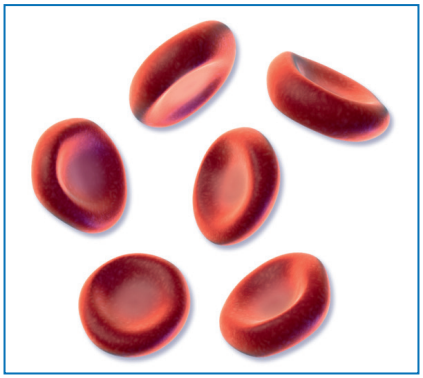

Figure 1. Red Blood Cells (erythrocyte) (3)

Their main function is to mediate gas exchange (4). Hemoglobin is a protein providing this function and filling almost the whole content of the cell. Red blood cells bond oxygen in lungs mediated through hemoglobin and transport oxygen to the tissues and make the return trip taking carbon dioxide back to the lungs.

Red blood cells are produced in bone marrow as all the other blood cells. Reticulocyte is an intermediate nuclear cell which is observed during the maturation of erythrocytes. The elasticity of the cell wall of ageing red blood cells declines and cells are caught while filtered in the sinusoids during their passage through the spleen. Average life-span of red blood cells in blood circulation is 120 days.

Red blood cells are examined in blood count with the parameters such as RBC, hemoglobin (HGB), hematocrit (HCT), Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC)

The decrease in red blood cell count is called anemia. Oxygen-carrying capacity of blood declines as a result of anemia. The case where an increase in red blood cell count is observed is polycytemia; polycytemia is observed in myeloproliferative disorders.

Hemoglobin: The functional protein which is found most frequently in red blood cells is hemoglobin. The most crucial function of hemoglobin is to mediate gas exchange between lungs and tissues as well as some other functions such as maintaining acid-base balance, transportation of nitric oxide (NO). More than 90% of the cytoplasm of a red blood cell is filled with hemoglobin. This protein with a molecular weight of 64.000 Dalton is a tetramer composed by the combination of four globulin chains containing one heme loop each. Heme is the functional part of hemoglobin composed of protoporphyrin IX ring in the middle of which an iron atom is found. Hemoglobin is the most important part of blood count because it is used both alone and in the measurements of many red blood cell indicators (5).

White Blood Cells (WBC, leukocytes):

White blood cells are called so because they have achromatic vision compared to red blood cells under the light microscope. They are divided into five different types.

Neutrophils: Neutrophiles which are an important component of hereditary immunity, have crucial functions in defense against microbial infections. 50-70% of white blood cells in blood circulation are neutrophiles and their count is 1.7-7.5 10^3/μL' on average.

They stain neutral pink in hematoxylin and eosin. Two types of granules are specified inside the neutrophiles. Primary granules are also known as azurophilic granules; they contain enzymes such as myeloperoxidase, elastase, proteinase. Secondary granules (specific granules) contain enzymes such as phosphatase, NADPH oxidase, collagenase. Nuclei of the neutrophiles of which the diameter varies between 12-15 μm have 2 to 5 segments (lobed or segmented). Therefore, they are denominated as polymorphonuclear neutrophil cells (PMN) together with eosinophiles and basophiles of which the nuclei are seen lobed under the microscope (4).

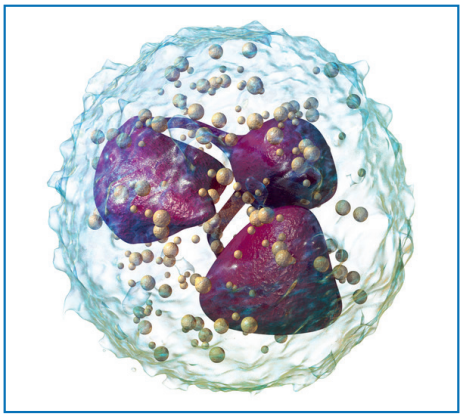

Figure 2. Neutrophiles (3)

Eosinophiles: They make just 1-3% of white blood cells; their diameter varies between 12-17 μm. They stain well with acidic dyes; they are observed dark red under the light microscope. The structures that stain are granules; they are full of enzymes such as lipase, DNAse, plasminogen. Their particular function is to defend the body against parasitic infections (4). Eosinophile count also increases in allergic conditions.

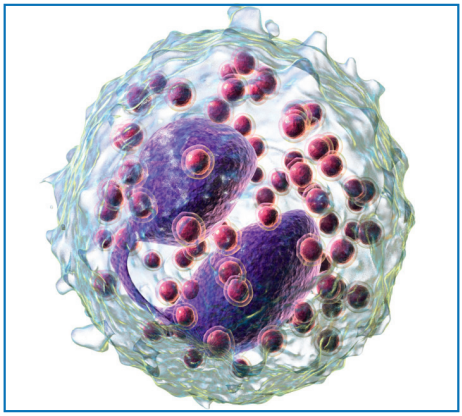

Figure 3. Eosinophile (3)

Basophils: Just 0.5-1% of white blood cells is basophile in blood circulation; the diameter of a basophile is 12 μm in average. They are called as basophiles due to well staining with alkaline (basic) dyes; they are blue under the light microscope. Molecules like histamine and serotonin make the content of the granules. Particularly in allergic conditions, the count increases (4).

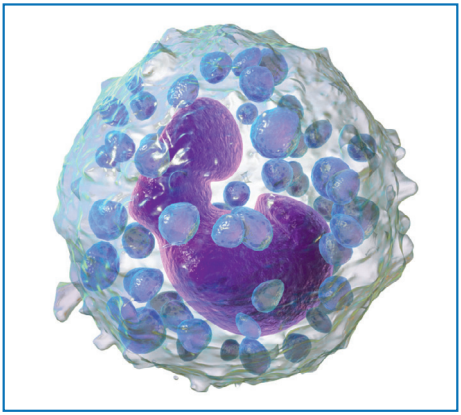

Figure 4. Basophile (3)

Lymphocytes: Lymphocytes make about 20-40% of white blood cells; its blood count is 1.0-3.2 103/μL. They are divided into sub-typed such as T cells, B cells, natural killer cells (NK). These cells functioning both in hereditary immunity and acquired immunity mediate almost all defence incidents of the immune system. Under the light microscope, the nucleus of these cells having a 7 μm size in average fills almost the whole of its cytoplasm; however, in the cytoplasm of some NK cells, granules can be observed (4).

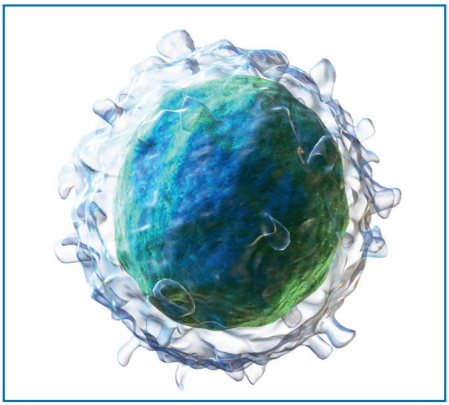

Figure 4. White Blood Cell (Lymohocyte) (3)

Monocytes: Monocytes are the precursors of the tissue macrophages in blood. They make 2-10% of white blood cells. They are the largest cells in blood circulation with their 12-20 μm diameters. Under the light microscope, their horseshoe-like nuclei is observed (4).

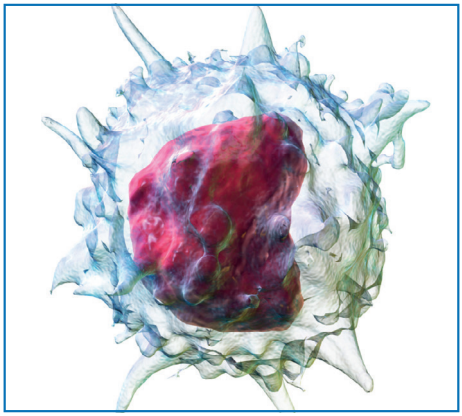

Figure 5. Monocyte (3)

Thrombocytes (platelet (PLT), blood-platelets): There is no nucleus in thrombocytes derived from megakaryocytes. The function of thrombocytes which are 2-3 μm in diameter is to plug holes by clotting where endothelial integrity is interrupted. For this reason, when necessary, they must be activated. Availability of two kinds of granules inside thrombocytes: While coagulation factors such as fibrinogen are found in alpha granules, delta granules is full of substances such as ADP, calcium, serotonin (4).

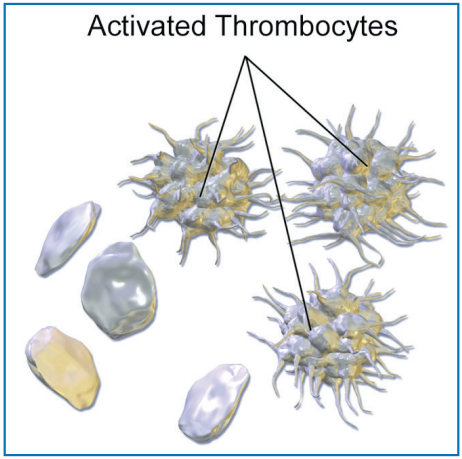

Figure 6. Thrombocytes (3)

2.2. Development of Complete Blood Count Method:

Blood count measurement is one of the oldest laboratory methods providing to evaluate blood cellular components both quantitatively and qualitatively. At present, a complete blood count is one of the most frequently requested tests.

The one who firstly specified blood count as a test that helps to the clinical diagnosis of diseases is Karl Vierordort (1818-1884). He investigated the blood by performing blood smears to be observed under the microscope (6). Thereafter, Luis Charles Malassez (1842-1909) influenced on the advancement of blood count method with his studies. He facilitates blood count by designing a new mechanism providing whole blood to become diluted (7).

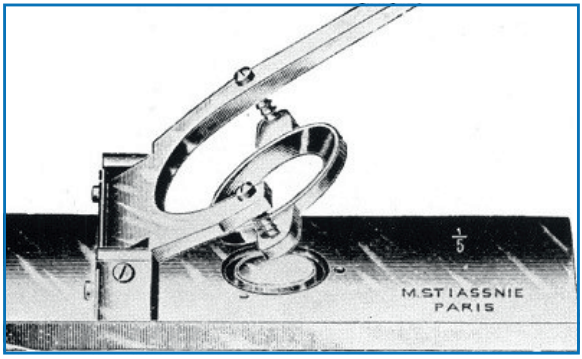

Figure 7. Blood count instrument developed by Malassez (Hemocytometer) (6)

One of the most important developments in blood count is realized by Richard Thoma (1847-1923). He applied complete blood count specimen into the hallow which is found on the slide that he designed specifically and has 1 mm sides, 0.1 mm depth and a shape of tetragonal prism with a volume of 0,1 mm3, by diluting the specimen with his specific pipette. In Thoma slide, the counting area is divided into 16 big squares, and these squares are divided into 400 little squares (Figure 8). Blood cells can be counted through Thoma slide under the microscope. Thoma slide is manufactured commercially by the firm Carl Zeis and launched to the daily laboratory consumption (8).

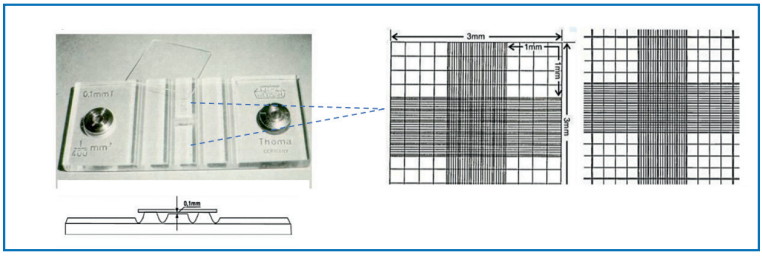

Figure 8. Thoma slide (8)

At the beginning of 1900s, it was seen that Thoma slide has insufficient performance for white blood cell count and then researchers such as Bauer, Türk, Bürker developed white blood cell count further, also by the help of diverse staining methods (6).

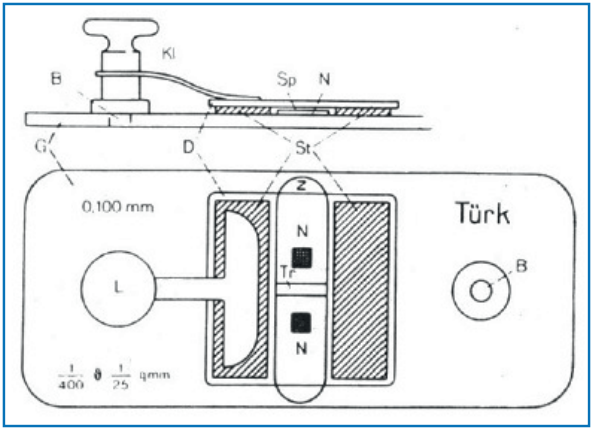

Figure 9. Blood count apparatus developed by Bürker and Türk (Hemocytometer) (7)

Until the 1950s, blood count remained to be a manual procedure, but after then the need of automatic cell count method gradually increased due to the increase in the workload of laboratories. At this point, the big revolution became true by courtesy of Coulter Brothers. Coulter Brothers developed the application which is known as the Coulter Method at present.

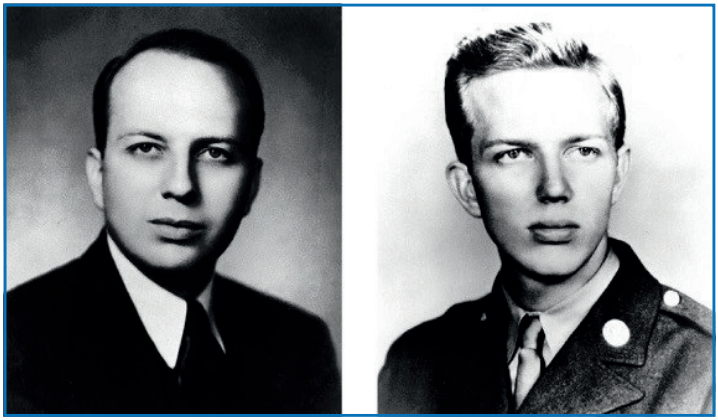

Figure 10. Coulter Brothers. Wallace H. Coulter on the left, Joseph R. Coulter on the right (9)

Figure 11. Example of the first automatic blood count device developed by Coulter Brothers (9)

After 1980, computerized automatic blood count devices took the place of blood count which was performed under the microscope manually. Automatic blood count devices have provided to measure or calculate at least 18 parameters in whole blood samples having a volume lower than 200 µL within a time period such as 1 minute. Although there are a lot of blood count devices from different brand in the market, almost all of them operate with the similar methods.

3. PRINCIPLES OF THE MEASUREMENT IN AUTOMATIC BLOOD COUNT DEVICES

3.1. Impedance Method in Blood Count (Coulter Method)

Coulter Brothers observed that as blood cells suspended in an ionic solution are directed to pass through a narrow space, they cause changes in electric current sensed by the electrodes placed in the space according to the type of the passing cells. Coulter Brothers, in their device, found out that there happened to be an alteration in lower frequency electric current applied between two electrodes placed inside and outside the tube when blood cells were passing respectively through the space (aperture) made on a glass tube (Figure 12). As each cell passes through the aperture, change in the electrical the resistance between these two electrodes, alias impedance, caused the alteration (10).

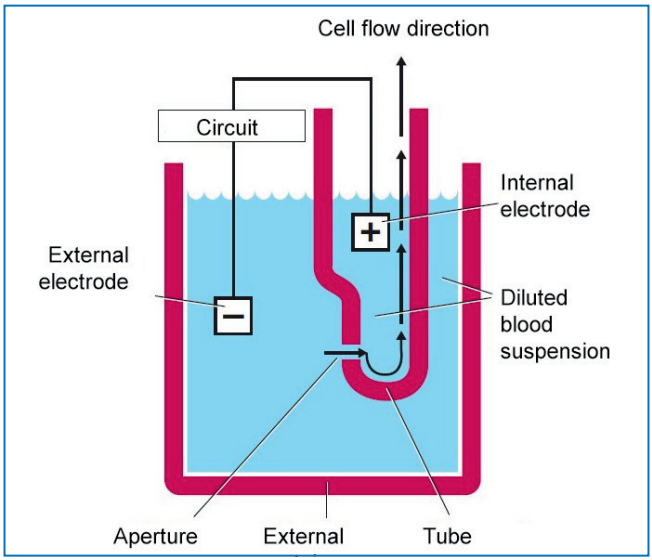

Figure 12. The general design of automatic blood count devices running according to the method of impedance (10,11)

In Coulter device, each beat (peak) read on the oscilloscope is counted as a cell passing. Magnitude in voltage change is proportional to the size of the cell passing at that moment.

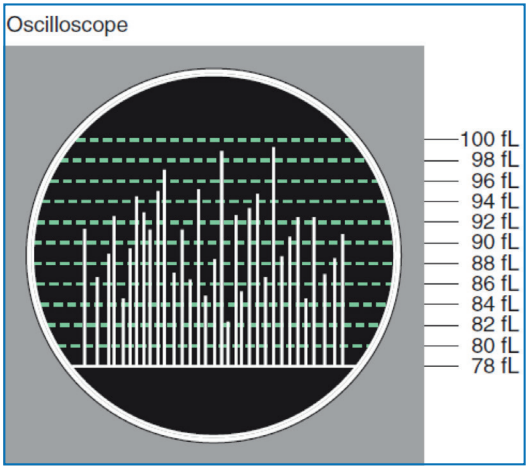

Figure 13. Counting blood cells in oscilloscope in automatic blood count devices. Each peak line shows a cell passing and the height of the peak bar shows the size of the cell in femtoliter (fL) (10, 11)

Cell counts together with their sizes are added into a histogram graphic. In these graphics, cell populations can be differentiated with certain threshold values. Thus, both counts and sizes of the blood cells are measured (Figure 13).

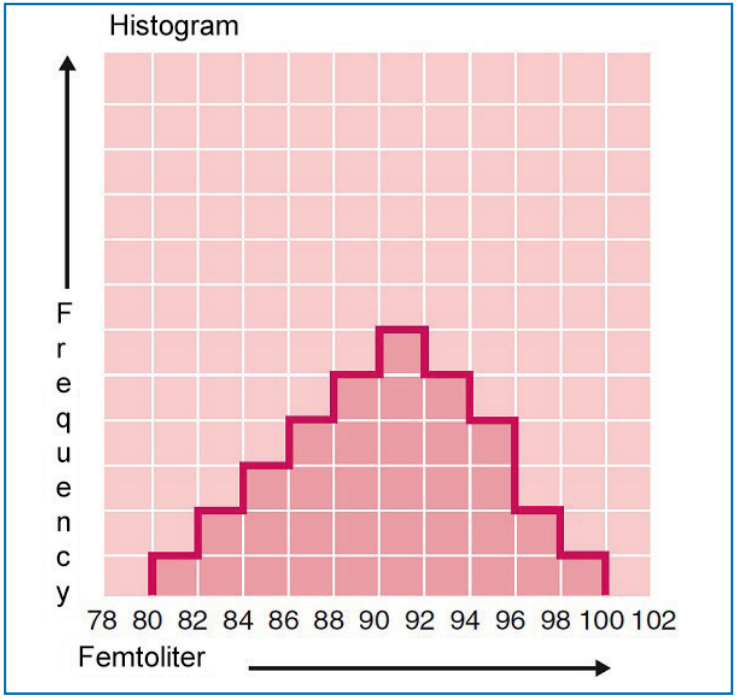

Figure 14. Presentation of the distribution of blood cells counted by automatic blood count devices according to the size of the cells (10,11)

3.2. Flow Cytometry Method in Blood Count

Although the Coulter method successfully counts red blood cells, it has not displayed the success expected for the white blood cells. This issue managed to be resolved by combining flow cytometers with blood count analyzers. With flow cytometer, size, shape, biochemistry or antigenic components of a single cell can be determined. The light source in flow cytometers is laser. As the cell flows through the channel, exposure of the cell to the laser light causes light scatter. Receptors placed in different angles can determine the intensity of the scattered light. Data coming from different angles are assessed and pooled and added to the scatter graphic (Figure 15). Thus, the type of white cells can be specified.

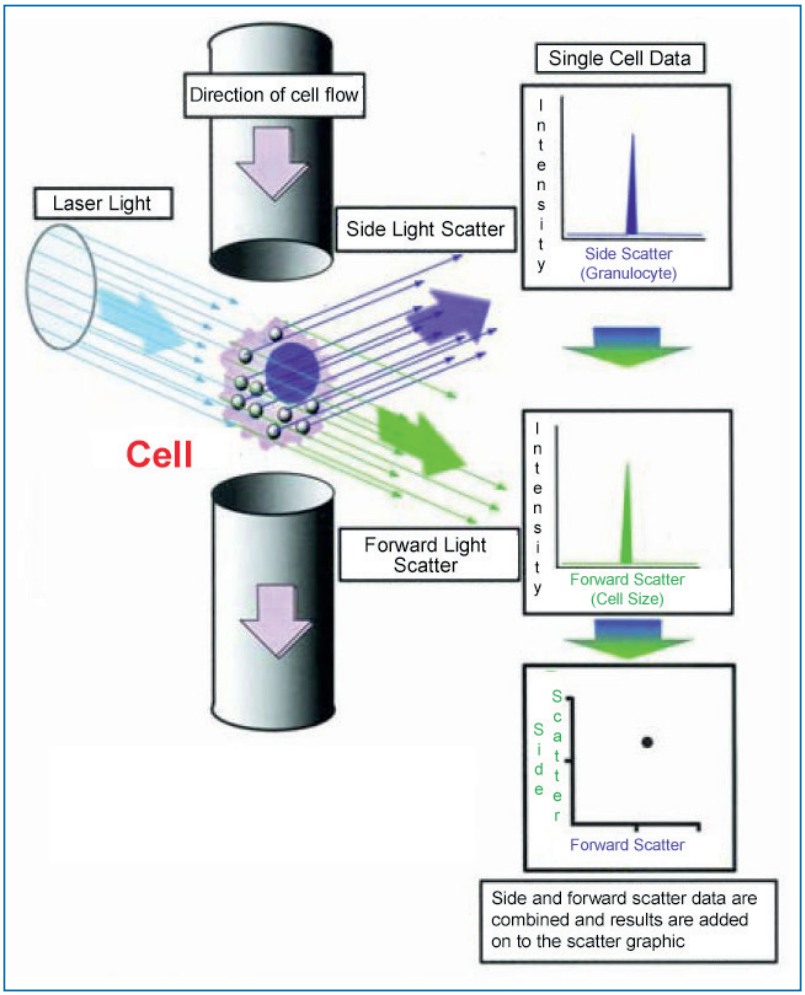

Figure 15. Specification of the type of white blood cells by flow cytometer (12)

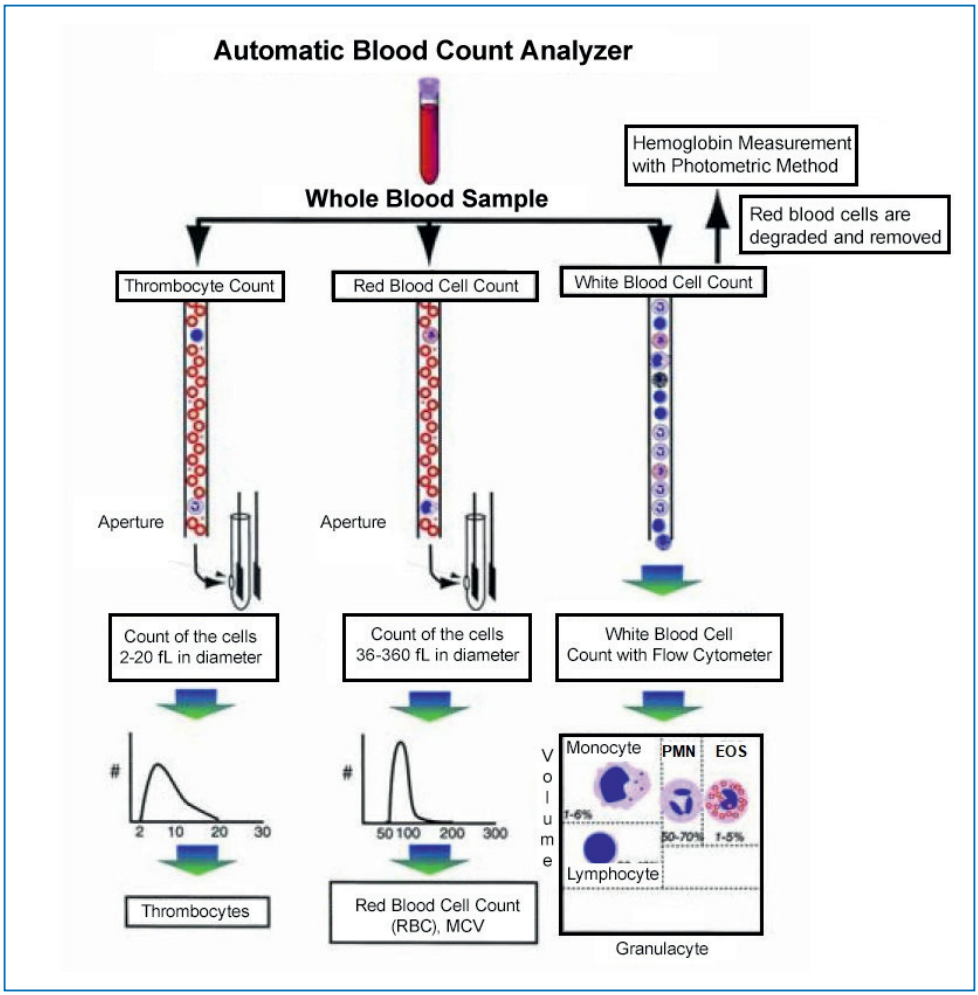

Figure 16. The general design for automatic blood count by the manufacturers (12)

3.3. Calculation of Complete Blood Cell Count Parameters

The complete blood count is developed by combining techniques more than one. At present, this development has been going on by combining new techniques with the techniques which are in use. In complete blood count, the initial technique used was a visual examination of the cells via microscope. HGB levels measured spectrophotometrically by cyanohemoglobin method (Drabkin method) enabled parameters known as red blood cell indicators (erythrocyte indices) to be calculated. At present, photometric HGB measurement is an integral part of the automatic blood count analyzers.

Red Blood Cell Count (RBC), MCV: Red blood cell count is reported as RBC. Size of the red blood cells is also essential as their count. Mean corpuscular volume (MCV) is an auxiliary indicator, particularly in the differential diagnosis of anemia. In measurements performed under a microscope, MCV value is calculated by dividing measured hemotocrite value (HCT) by red blood cell count (RBC).

HCT: In this context, another critical indicator (parameter) is the HCT value. HCT is the proportion of total cellular components in the blood to total blood volume in percentage. Before the development of automatic methods, HCT levels used to be measured in the way that blood samples were collected in capillary tubes and these capillary tubes were resolved using micro-centrifugation. However, with the development of automatic blood count, HCT has become a value calculated by using RBC counts and sizes instead of being a measured value (Figure 17).

$$MPV(fL) = \frac{PCT}{PLT}$$

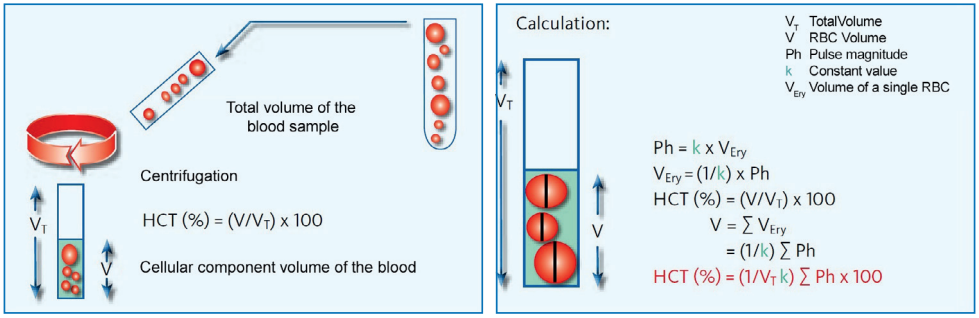

Figure 17. On the left; hematocrit measurement using of micro-centrifugation (HCT (%)). On the right; calculation of hemotocrit in automatic blood count analyzers (13)

MCH, MCHC: Another two important red blood cell indicators are MCH and MCHC. MCH and MCHC serve in specifying the type of anemia. When MCHC value drops below 32, red blood cells are denominated as hypochromic because of the faint appearance of red blood cells due to iron deficiency, but not the other way round; in fact, under physiological conditions, MCHC level in red blood cells do not rise over 36. Hyperchromia definition, which is a microscopic assessment is a misattribution. In such cases, for the structure of red blood cells changes and turns into spherocyte, they are seen much more filled and darker in color. Therefore, they have been specified as hyperchromic. MCHC value changes a little bit lifelong of an individual. There are minimal factors that affect MCHC level. Therefore, MCHC is an auxiliary indicator, particularly in evaluating preanalytical errors.

$$MCH\ (pg) = \frac{HGB}{RBC}$$

$$MCHC\ (g/dL) = \frac{HGB}{HCT}$$

RDW: It is a quantitative measurement of differences among red blood cell sizes. The distribution of red blood cell sizes is calculated by added on the histogram (Figure 18). Results of the patients are reported in standard deviation (RDW-SD), variation coefficient (RDW-CV).

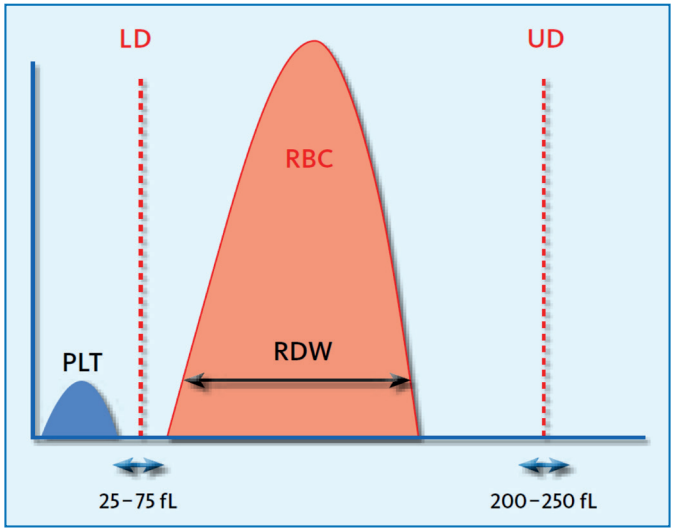

Figure 18. Calculation of RDW by histogram (13).

*__LD:__ Lower detection limit, __UD:__ Upper detection limit

White Blood Cell Count (WBC): High count of red blood cells in a blood sample conceals white blood cell count. Therefore, for blood count analyzers to perform the measurement, red blood cells are removed by lysing red blood cells with substances in surfactant specifications (lyse solution). Then, white blood cells are counted directly according to the measurement method of the manufacturer company. In the patient's results, white blood cell count (WBC) in total and sub-component of each white blood cell are reported both in count and in proportion (Table 2). In designs of blood count analyzers launched by different manufacturers, changes can exist in measurement methods and calculations. In Table 2, measurement and calculation methods of the most frequently used blood count analyzers are summarized.

Platelet (thrombocyte) count: Platelet count can be more difficult than other cells due to their small dimensions. In addition, while residual pieces of other degraded cells, micro-erythrocytes and bacteria lead to false-high platelet counts, giant platelets or platelet aggregates lead to false-low platelet count values. In patient's results, platelet values are reported together with platelet distribution width (PDW), MPV and plateletcrit (PCT) obtained by dividing platelet volume by platelet count.

Tablo 1. Measurement methods of blood count parameters by different manufacturers

Parameter	Beckman Coulter UniCel DxH 800	Sysmex XN Series	Abbott CELL-DYN Sapphire	Siemens ADVIA 2120i	Mindray BC Serie
WBC	Impedance	Fluorescent stained light scatter	Light scatter	Light scatter	Fluorescent stained light scatter
RBC	Impedance	Impedance	Impedance	Laser light scatter	Impedance
HGB	Cyano hemoglobin 525 nm	Sodium lauryl sulfate 555 nm	Cyano hemoglobin 540 nm	Cyano hemoglobin 546 nm	Photometric measurement without cyanide
HCT	(RBC x MCV)/10	Total RBC peak magnitude	(RBCx MCV)/10	(RBC x MCV)/10	(RBC x MCV)/10
MCV	Average obtained from the RBC diameter distribution histogram	(Hct/RBC) x 10	Average obtained from the RBC diameter distribution histogram	(HCT/RBC) x 10	(HCT/RBC) x 10
MCHC	(HGB/HCT) x 100	(HGB/HCT) x 100	(HGB/HCT) x 100	(HGB/HCT) x 100	(HGB/HCT) x 100
Reticulocyte	Supravital staining light scatter	Fluorescent stained light scatter	Supravital staining light scatter	Fluorescent stained light scatter	Fluorescent stained light scatter
PLT	Light scatter impedance in common	Light scatter impedance in common	Light scatter impedance in common	Light scatter	Impedance

Tablo 2. Reference ranges, units, measurement methods and formulas of blood count parameters

Parameter	Reference range	Unit	Measurement method	Formula
Red blood cell indicators				
RBC	Female:3.8-5.2 Male: 4.2-6	10^6 /µL	Count	
HGB	Female:12-15 Male: 13.5-18	g/dL	Photometric measurement	
HCT	Female: 35-49 Male: 40-54	%	Calculation	
MCV	80-100	fL	Calculation	$MCV(fL) = \frac{HCT}{RBC}$
MCH	26-34	pg	Calculation	$MCH(pg) = \frac{HGB}{RBC}$
MCHC	32-36	g/dL	Calculation	$MCHC\ (g/dL) = \frac{HGB}{HCT}$
RDW	11,5-14,5	%	Calculation	Histogram
Platelet parameters				
PLT	150-450	10^3/µL	Count	
MPV	7-12	fL	Calculation	$MPV(fL) = \frac{PCT}{PLT}$
White blood cell parameters				
WBC	6.3-10.6	10^3/µL	Count	
Neutrophil	1.7-7.5	10^3/µL	Count	
Neutrophil #	50-70	%	Calculation	
Lymphocyte	1-3.2	10^3/µL	Count	
Lymphocyte#	18-42	%	Calculation	
Monocyte	0.1-1.3	10^3/µL	Count	
Monocyte#	2-11	%	Calculation	
Eosinophil	0-0.3	10^3/µL	Count	
Eosinophil#	1-3	%	Calculation	
Basophil	0-2	10^3/µL	Count	
Basophil#	1-2	%	Calculation	

4. VENOUS BLOOD COLLECTION FOR COMPLETE BLOOD COUNT

4.1. Preparing the Patient

4.1.1. Posture

Switching to sitting position or upright position from prone position causes fluid flow from the vessels into the interstitial space. It is known that, as a result of this fact, molecules which cannot diffuse into the tissue due to their dimensions such as blood cells, proteins, cholesterol and iron are measured high. It is reported that HGB values increase by 8%, RBC values by 8% and WBC up to by 15% between supine position and sitting or prone position of the same person (14).

4.1.2. Exercise

Exercise can affect analytes such as creatinine, creatinine kinase, myoglobin, aspartate aminotransferase as well as the results of complete blood count (15). It is reported that WBC, neutrophil and lymphocyte values increase significantly and high neutrophil count lasts more than 2 hours after heavy exercise (16).

4.1.3. Circadian Rhythm

It has been known that circadian rhythm has an impact on blood count. Although RBC, HGB and HTC display a little increase at 11:00 in the morning, an increase in leukocytes can be observed in the evening between 21:00-24:00 (17). It is reported that while lymphocyte, eosinophil and basophil counts reach their highest value, they decrease in morning hours. This change is inversely related to the cortisol level (18). It is reported that PLT count increases in the evening and decreases in the morning (19). For standardization in results, it is appropriate to perform blood sampling in the morning.

4.1.4. Stress

Anxiety and particularly crying of children during blood collection can cause increase in leukocyte count (15).

4.1.5. Diet

Blood should be drawn for sampling after at least 8-12 hours fasting period (15). Glucose and lipid content of the blood increase in individuals who eat within 2 hours or less before drawing the blood sample. It can affect MCV, MCHC, HCT results in high concentration. Excessively high lipid content can lead to interference in tests where photometric measurement is performed such as hemoglobin (17). In addition, it has been reported that when blood glucose level increases over 500 mg/dL, MCV value is affected due to the osmotic impact (20).

4.1.6. Smoking

Before blood sampling, smoking can cause an increase in leukocyte count. The long duration of smoking causes increases in hemoglobin level (15).

Recommendations:

- 8-12 hours fasting before blood sampling is recommended.
- Heavy physical exercise should be avoided within 24 hours prior to blood sampling.
- It is recommended not to smoke at least 2 hours before blood sampling for blood count.

4.2. Blood Sampling Equipment Used in Complete Blood Count

4.2.1. Blood Sampling Tubes

Plastic tubes are preferred because they are flexible, resistant to high centrifugation speeds and safer for the staff; in addition, they are adjustable so they ensure a decrease in medical waste burden and they do less harm in the environment.

Because of these reasons, plastic tubes are more frequently chosen and are used prevalently.

Plastic tubes are manufactured using polyesters such as polyethylene terephthalate (PET), polyolefins such as polyethylene and polypropylene (PP), polyacrylic, polytetrafluoroethylene, polysiloxane, polyvinyl chloride, polyacrylonitrile and polystyrene. However, plastic tubes have more gas permeability compared to glass tubes. PET which is non-breakable and ensuring longer vacuum retention is prevalently used in the production of blood sample tubes. And PP, for it has less water permeability, is another selected plastic material because it provides keeping the volume and concentration of liquid anticoagulation agents (21-24).

4.2.2. Tube Additives: Anticoagulants

The most critical point of blood collection in complete blood count is to draw the sample into the right tube. Drawing the sample into the EDTA tubes known to be ‘lavender cap’ in daily laboratory speech has become a standard for complete blood count analyses. That is the most basic information that must be well known by all healthcare workers taking roles in preanalytical phase. Thus, collecting blood sample in improper tubes, therefore sample transfer from tube to tube can be prevented.

Ethylenediaminetetraacetic acid (EDTA, $C_{10}H_{16}N_2O_8$); is specified as the most appropriate anticoagulant for hematological tests because it keeps both the morphology of blood cells and cellular content. For complete blood count, EDTA salts are used as an anticoagulant. EDTA salts, being a chelating agent, bind calcium in blood and inhibit coagulation cascade. As EDTA salts, K_3EDTA (tri-potassium EDTA), K_2EDTA (di-potassium EDTA), Na_2EDTA (di-sodium EDTA) can be used (25, 26).

K_2EDTA and Na_2EDTA are applied on the inner surface of the tubes sprayed and dried. But the advantage of K_2EDTA is that it is more soluble than Na_2EDTA. K_3EDTA is found in liquid form in the tubes. Although liquid EDTA has the advantage of mixing with blood and lack of clots, it can cause sample dilution due to its liquid form. Therefore, it is pointed out that directly measured values are resulted in lower levels by 1-2% compared to K_2EDTA adulterant blood samples. In addition, as EDTA concentration rises, K_3EDTA causes more shrinkage in RBCs due to excess potassium ions it contains (11% reduction in 7.5mg/mL blood). Therefore, K_3EDTA causes reduction also in MCV values (typically, a difference between -0.1% and -1.3% has been observed compared to K_2EDTA). However, it is observed that there is a much more increase in cellular volume in pending blood samples with K_3EDTA (1.6% increase after 4 hours). International Hematology Standardization Council and CLSI recommend K_2EDTA that should be used in the measurements of blood cell count and blood cell dimensions due to all of these reasons (26,27). K_2EDTA and K_3EDTA concentrations are recommended to be 1.5 – 2.2 mg/mL as an anticoagulant (27).

4.2.3. Insufficient Sample Volume

It has been reported that the second source of preanalytical error in complete blood count tests is the collection of insufficient blood sample (28-30). It has been stated above that two types of EDTA salt additives are used in the tubes used for drawing complete blood count sample. While K_2EDTA is sprayed onto the inner surface of the tubes in dry form, K_3EDTA exists in the tubes as liquid solution. Therefore, it is known that K_3EDTA tubes cause dilution in samples and that this leads to reading the results under the real values for all of the parameters that are measured by 1-2%. However, drawing blood in lower volumes than the volume recommended for K_3EDTA tubes will increase this dilution effect. Another negative effect of drawing blood in lower volume is the shrinkage occurring in blood cells due to hyperosmolarity in both K_2EDTA and K_3EDTA tubes. As a matter of this fact, it is reported that while a decrease in MCV and HTC values is observed, there is an increase in MCHC values (31-33).

At present, blood sampling tubes, their holders and needles are used together providing a system. In evacuated blood sample tubes, it is specified how much volume of blood shall be drawn following vascular access. All manufacturers put a fill line indicator on the tubes in order to observe this volume (Figure 19). In order to provide the right blood/anticoagulant ratio which is needed for correct test results, it should be paid attention to drawing the volume of blood specified by the manufacturer company (fill line). The overall approach is in the direction that the tube can be filled with the sample about +10% of the tube's fill line (90%-110%) (34). For the speed of tube fill can vary between different brands, waiting until the end of the blood flow into the tube (till the vacuum exhausted) is important for sufficient blood drawing.

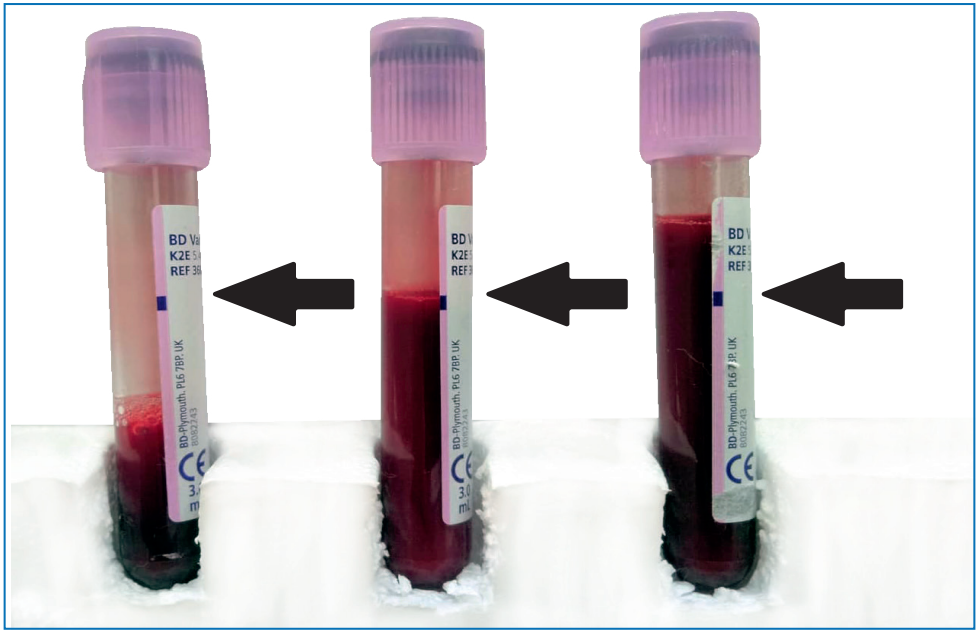

Figure 19. On the left, sample drawn in insufficient volume; in the middle, sample drawn in appropriate volume; on the right, sample drawn in excess volume

4.2.4. Blood Sampling Needle

In venous blood sampling, the size of the sampling needle should be specified according to the amount of blood to be drawn, the age of the patient and the diameter of the vein. For drawing blood from the antecubital vein, 19-21 G (gauge) needles are ideal; smaller needles can be used in newborns and in adults who have thinner veins. It should be kept in mind that large G size numbers connote needles with small diameter and small G size numbers connote needles with large diameter.

4.3. Venous Sampling for Complete Blood Count

4.3.1. Blood sampling staff

It has been recommended that venous sampling should be done by trained nurses and phlebotomists for all tests. The staff that will draw blood for sampling should be trained especially about tube sequence, specifications of the equipment to be used and tube filling volume.

4.3.2. Order of Draw for EDTA tubes

In patients for whom the EDTA sample is ordered together with the other laboratory tests, the order of draw of the tubes that have different specifications is crucial. After venipuncture, drew order stated in the Guidelines for Venous Blood Sampling should be followed (Table 3) (35).

Tablo 3. Order of draw and mixing to comply with for sample tubes according to the features of the tests ordered (35).

Cap color	Tube/Additive	Mixing
Parameter (1)	Blood culture/Medium	In order to make medium and blood mix, it is inverted slightly
(3)	Coagulation tube / Citrated	3-4 times
(4)	ESR tube / Citrated	3-4 times
(5)	Serum tube / Without gel	5 times
(5)	Serum tube / With gel	5 times
(5)		5 times
(5)	Serum tube / Tube with thrombin clot activator	5 times
(6)	Plasma tube / Heparin tube with or without gel	8-10 times
(7)	Plasma tube / EDTA tube with or without gel	8-10 times
(8)	Plasma tube / Fluoride / Potassium Oxalate: Fluoride / EDTA Fluoride / Heparin	8-10 times

Warning: Tubes should be filled until the vacuum exhausted and blood flow stops. Tubes containing additives (such as EDTA, citrate, heparin) should be filled up to the volumes recommended by the manufacturer or until ensuring the correct blood/additive ratio.

***EDTA;** ethylenediamine tetraacetic acid, **ESR;** erythrocyte sedimentation rate

4.3.3. Mixing the EDTA tube

In addition, all tubes independently of EDTA salt used for anticoagulation should be inverted 8-10 times in order to ensure a complete mixture of anticoagulant and blood. Doing this procedure imprecisely is one of the most essential preanalytical error sources, imprecisely. This procedure should be repeated 8-10 times as shown in Figure 20. Tubes must not be shaken, must be inverted gently.

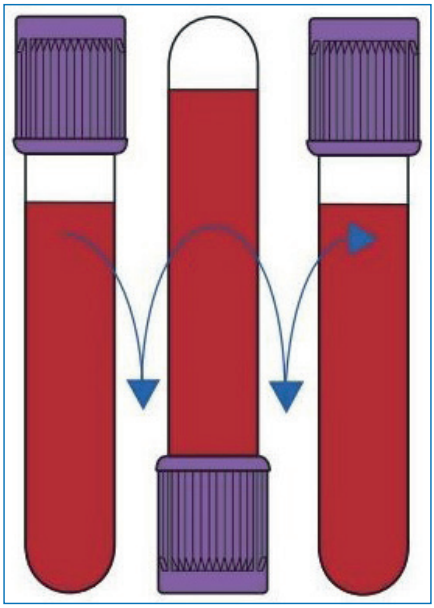

Figure 20. Mixing the whole blood sample collected in EDTA tube by inverting

4.3.4. EDTA-dependent pseudothrombocytopenia

It is considered that autoantibodies against glycoprotein originated as a result of an interaction between EDTA and the glycoprotein IIb-IIIa found on

the platelet membrane leads to platelet aggregation. Consequently, pseudothrombocytopenia is specified. Prevalence of EDTA-dependent pseudothrombocytopenia is reported to be 0.1%. If such a condition is suspected, the test is repeated after collecting a blood sample in another tube containing an anticoagulant other than EDTA (such as sodium citrate, heparin). If the case is EDTA-dependent pseudothrombocytopenia, then platelet count is expected to be corrected (35). Besides, the platelet count can be controlled by peripheral smear with a finger stick capillary sample.

4.3.5. Effect of Blood Sampling Procedures

At every stage of:

- Identity validation,
- Choosing and preparing the equipment that will be used for blood collection,
- Choosing vascular access site (vein),
- Cleaning vascular access site,
- Tourniquet duration,
- Venipuncture and blood collection,
- Tube sequence

which are applied during blood collection in the patient, it should be comply with related guidelines (35-37).

Tourniquet application should not exceed one minute for it can locally halt circulation system associated with hemoconcentration and infiltration of blood to tissue. If tourniquet application exceeds this period of time, analytes having a protein structure, cellular blood volume and other cellular element levels are found to be erroneously high. Among the parameters affected, along with analytes such as albumin, potassium and calcium, complete blood count parameters such as RBC, WBC, HGB, HCT take place. It is reported that if tourniquet duration lasts for 2 minutes, there is a significant increase in HGB and HCT values (36). In order to prevent this case, it is recommended to loosen the tourniquet and reapply after two minutes if it is applied for more than one minute (38).

4.3.6. Blood volume

Blood volume collected should be so as to minimize iatrogenic (related to phlebotomy) anemia risk particularly in pediatric patients and individuals with

any critical illness. In order to prevent iatrogenic anemia, total blood amount drawn from the patient should be monitored and limited on the base of the time period.

In references, blood volume for children 75-80 mL/kg and for newborns it is higher. And in adults, it is specified as 65-70 mL/kg. It is recommended to limit so as not to exceed 1-5% of the total blood volume within 24 hours and not to exceed 10% of total blood volume within 8 weeks (38). Also, EDTA tubes with lower volumes providing to draw lower volumes of blood.

Blood amount filled into the tube is also essential. If it is overfilled, blood is not well mixed with the anticoagulant in the tube, and this can lead to erroneous test results (25).

Recommendations:

• For blood count tests, blood is drawn into the tubes with lavender cap (EDTA additive).

• Blood is to be drawn in the proper and appropriate volume. In order to ensure proper blood/anticoagulant ratio, the tube should be filled up to the fill line on the tube pointed out by the manufacturer. Much or less blood should not be collected (Figure 19).

• Recommended equipment should be used; blood should not be drawn definitely using a syringe.

• In venous sampling, phlebotomists should determine the gauge of the blood collection needle according to the blood volume that will be drawn from the patient, his/her age and the diameter of vein of the patient. 19-21G size numbered blood collection needles should be used for blood count tests. For children or and individuals with thin veins, small size numbered needles (>21G) can be used.

• Blood transfer from tube to tube must not be done.

• After venipuncture, one should be comply with the blood sampling sequence stated in the Guidelines for Blood Sampling (Table 3) (35).

• The tube should be inverted 8-10 times in order to ensure blood and anticoagulant is completely mixed (Figure 20).

• For all tests to be performed, it is recommended that venous blood sampling should be done by trained nurses, phlebotomists. These staff should be trained in regular intervals

5. SAMPLE TRANSPORTATION

Samples should be held on the tube racks straightly; swinging and shaking should be avoided as possible as it can be. Specimens should be transported in nested special primary (sample tube), secondary (in a structure that can prevent infectious contamination even if the cap is opened and containing sorbent material) and tertiary (can be used as transportation bag, protected against temperature changes) containers so as to control heat. Heat control can be performed using devices that record temperature changes. If the samples will be tested distant laboratories, refrigerated transportation is the recommended application. On the other hand, it should be kept in mind that keeping samples in the refrigerator can stimulate coagulation and these samples should be evaluated with regards to the presence of coagulum.

6. SAMPLE STORAGE

6.1. Time Period of Complete Blood Count and Conditions of Sample Storage

If samples of complete blood count analyses are kept waiting at room temperature, it is recommended to perform the tests within 6 (six) hours just after blood drawing (30). If samples cannot be tested within 6 hours, they can be kept in the refrigerator at +4°C for 24 hours. In samples kept in the refrigerator, it is reported that MCV, HCT, WBC values are more stable (39-42).

Recommendations:

• If samples of complete blood count tests are kept waiting at room temperature, it is recommended to perform the tests within 6 (six) hours just after blood drawing.

• If samples cannot be tested within 6 hours, they can be kept in refrigerator at +4°C for 24 hours (38).

7. CRITERIA OF SAMPLE REJECTION

Many of the preanalytical variables affecting complete blood count can be controlled. In case of complying with the blood collection instructions stated in the previous article, a whole blood sample which is appropriate for blood count can be obtained. However, getting an appropriate sample, meeting the required features, may not be always provided. Recognizing such cases, if needed, this sample or results obtained from these samples should be rejected.

Research concerning this issue suggests similar data. According to these research, preanalytical errors that are observed most frequently in blood count tests are listed below (30,44,45):

- Clotted sample 57%
- Insufficient sample 14%
- Improper tube 7%
- Hemolysis 2.7%
- Lipemia 2.3%

7.1. Clotted samples

Regarding complete blood count test, the most observed preanalytical error source is reported to be the clotted samples (28,29,45).

Potential factors that cause clotted samples:

- Insufficient mixing of blood and the additive just after the sample collection,
- Difficult blood drawing,
- Storing the sample in the refrigerator,
- Collecting blood sample over or below the fill line indicated by the manufacturer,
- Blood drawing by using a syringe,
- Blood transfer from tube to tube (especially from the tubes containing coagulant to EDTA tubes).

Removing the clot using tools like wooden applicator from the tube is a frequent misapplication in laboratories and it is not recommended definitely. This application can lead to hemolysis and false low values in results of all parameters. Clot residues in the sample can cause occlusions in the probes and tubing of analyzers, and if it escapes the attention, it can cause interferences during measurement.

Recommendations:

• If clotting is observed in a whole blood sample, this sample must be rejected definitely (28-30,44). This application can lead to hemolysis and false low values in the results of all parameters.

• Removing the clot using tools like wooden applicator from the tube is a frequent misapplication in laboratories and it is not recommended definitely.

It is easy to detect large clots by the naked eye. At present, there are clot detectors in almost all blood count analyzers found in laboratories. Even if a clotted sample is applied to the device, it will not work, but micro-clots may not be detected by the detector. In the presence of clots in the sample, for blood cells are wrapped with clots, it can be observed false low results of all parameters. In such a case, low MCHC values can be a warning.

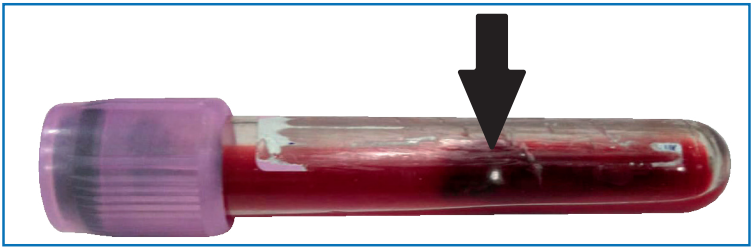

Figure 21. A clot in a whole blood sample

7.2. Hemolysis

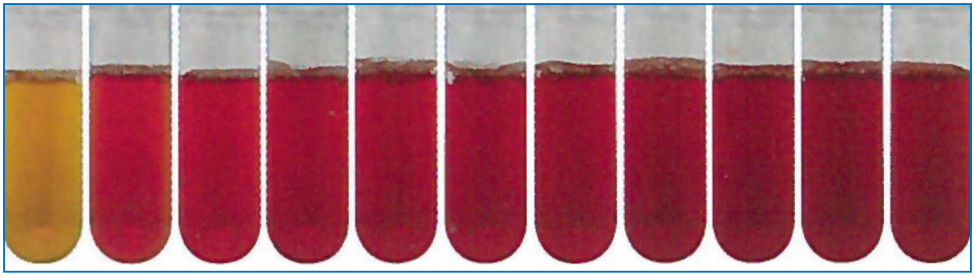

Figure 22. Visual evaluation of hemolysis (46)

Hemolysis is the degradation of red blood cells in vitro or in vivo. However, most of the hemolysis events occur due to the mechanical impairment of the the integrity of red blood cells during sample collection, transportation, processing or storage. Hemolysis is a critical problem particularly in blood samples coming from the emergency service and in samples drawn from children (47).

Potential hemolysis causes:

- Difficult blood drawing,
- Blood drawn using a syringe,
- Prolonged tourniquet application,
- Small needle size (larger than 23 G),

- Hematoma formation,
- Sample contaminated by ethanol or water,
- Shaking the tube after blood draw or during transportation,
- Blood transfer from tube to tube,
- Insufficient blood draws into the vacuum tube.

Degradation of red blood cells within the sample is a part of hemoglobin measurement method (analytical phase), but the early occurrence of this event accidentally in the preanalytical phase causes particularly red blood cell parameters be counted or calculated improperly. While hemoglobin value is not affected, RBC value decreases due to the degradation of red blood cells, consequently HCT, MCV values will decrease and MCHC value will be calculated falsely elevated. Regarding complete blood count, it is very difficult to detect hemolysis in the preanalytical phase. In tests performed in serum or plasma, the presence of hemolysis can be detected visually or by analyzers and a flag can be formed.

An increase in platelet (PLT) values can be observed depending on hemolysis. The reason of this issue can be that degraded red blood cells residues are read as platelets (48). But hemolysis cannot be detected visually in a whole blood sample. Therefore, while evaluating the patient results, MCHC values can be warning.

7.3. Icterus (Bilirubinemia)

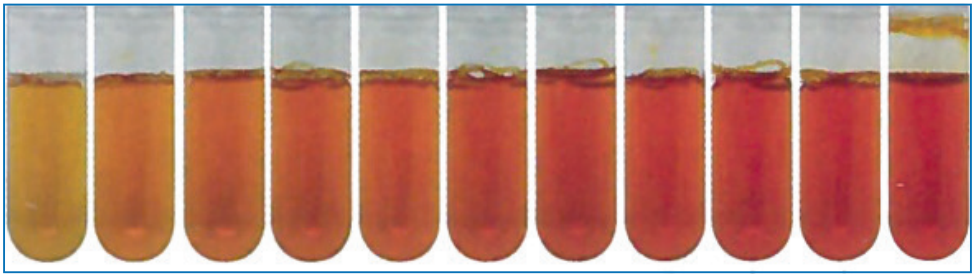

Figure 23. Visual evaluation of bilirubinemia (46)

High bilirubin value is an important interference reason. Bilirubin gives absorbance between 340-500 nm. For hemoglobin measurements are done in close spectrums, high bilirubin levels can cause interference. Depending on the high bilirubin levels, HGB can be read as falsely elevated; MCH values calculated from hemoglobin will also be found to be high (10, 49).

7.4. Lipemia

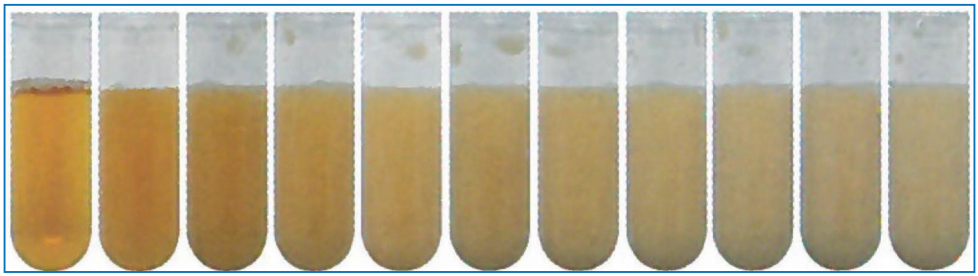

Figure 24. Visual evaluation of lipemia (46)

Regarding Turkish society, it is reported that triglyceride levels are over 150 mg/dL in 32% of female population and in 41.3% of male population (50). In postprandial samples, it is known that these values are found to be much higher. Lipemia over 300 mg/dL can be visible. Elevated triglyceride levels show the existence of chylomicron and VLDL of which the large part of the content is triglyceride increase in blood circulation. While the diameter of VLDLs from lipoproteins having particulate structure reaches 200 nm, chylomicron size reaches 1000 nm (51). In complete blood count of which measurement method is based on counting the particles, these lipoprotein particles can be counted as platelet, even as red blood cell or white blood cell leading to obtaining falsely elevated results. Nevertheless, the impact of lipemia is not limited to this. Lipemia affects the matrix of the sample, hence causes unwanted light scattering in photometric methods. By the blur it creates, in HGB measurement wavelength, it interferes with HGB causing to read falsely elevated hemoglobin values (52). In addition, lipoprotein particles cause analyte condensation in the sample by narrowing liquid compartment due to liquid excluding effect, hence lead to falsely elevated results in all of the analytes. Reflection of this fact on the blood count is again on hemoglobin values.

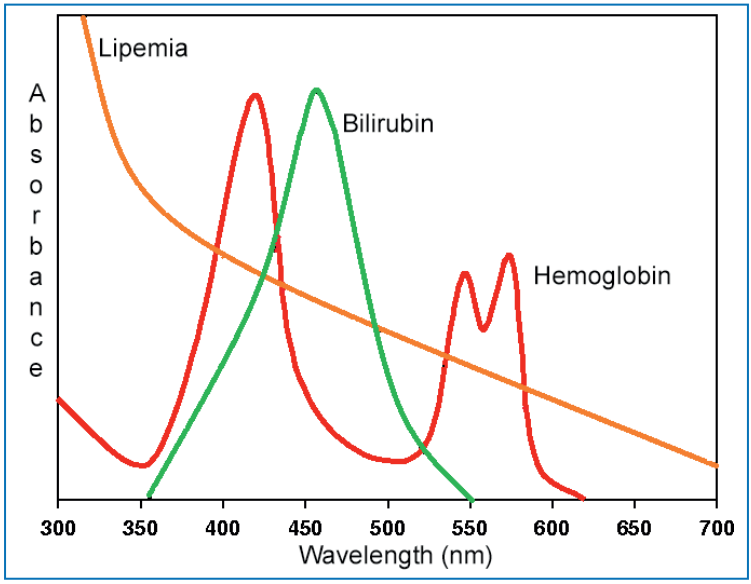

Figure 15. The absorption spectrum of lipid, bilirubin and hemoglobin (53)

Figure 22. Lipemic whole blood sample with EDTA (52)

7.5. Cold Agglutinins

Cold agglutinin disorder is an autoimmune disorder which is caused by antibodies generally in the type of IgM and sometimes IgA or IgG formed against polysaccharide antigens on the surface of red blood cells. In cold agglutinin disorder, antibodies that are activated by cold cause impairment in the red blood cell membrane and red blood cells are auto-agglutinated, if the case intensifies, it results in hemolysis. Its effect on complete blood cell count is similar to hemolysis; while RBC and HCT decrease MCHC increases. If a whole blood sample is kept waiting in 37°C water bath, agglutination clears up and real values can be reached in measurements performed once more again (55,56). If such a case is specified, it should be reported in patient results (45).

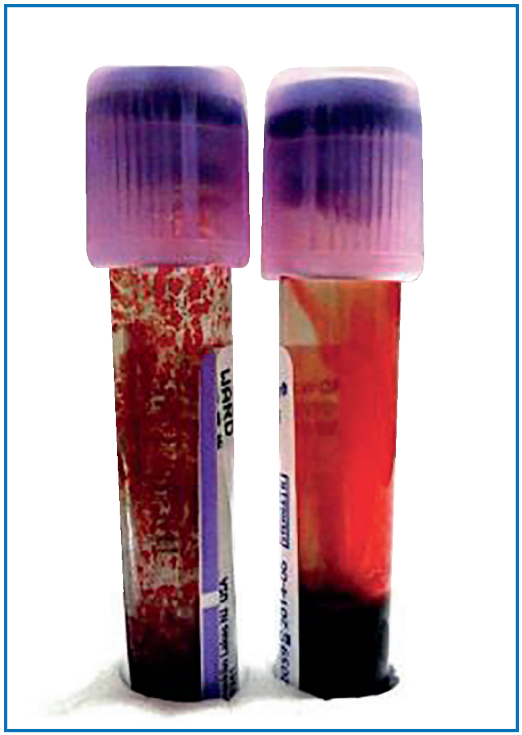

Figure 23. Visual comparison of a whole blood sample to control sample in cold agglutinin disorder (54)

Preanalytical error	Parameter affected in blood count	Cause	Recommendation
Clotted sample	No reading or decreases in all values in the presence of micro-clots	In the presence of clot residues, cells cannot be counted	Sample rejection
Hemolysis	RBC ↓, HCT↓	Red blood cells which are early degraded cannot be counted.	Sample rejection
Icterus	HGB↑, MCH↑	Photometric interference due to turbidity	Decision according to the patient's clinical status
Lipemia	HGB↑, MCH↑	Photometric interference due to turbidity	Decision according to the patient's clinical status
Red blood cells resistant to degradation	WBC↑,	In disorders such as hemoglobin S and C, red blood cells can be counted as white blood cell	Decision according to the patient's clinical status
Cold agglutinins	RBC↓, MCV↑, MCHC↑	Red blood cells aggregated together	Sample can be incubated in 37 °C and retested
Platelet aggregation	PLT↓, WBC↑	Aggregated platelets can be erroneously counted as white blood cells (EDTA interference).	Can be tested with a new sample drawn into another citrated, heparinized, etc. tube

Tablo 4: Frequently seen preanalytical errors, potential causes and solution recommendations

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